**Website address:**
https://www.gesundheitsindustrie-bw.de/en/article/news/enzyme-variants-designed-on-a-virtual-drawing-board



# Enzyme variants – designed on a virtual drawing-board

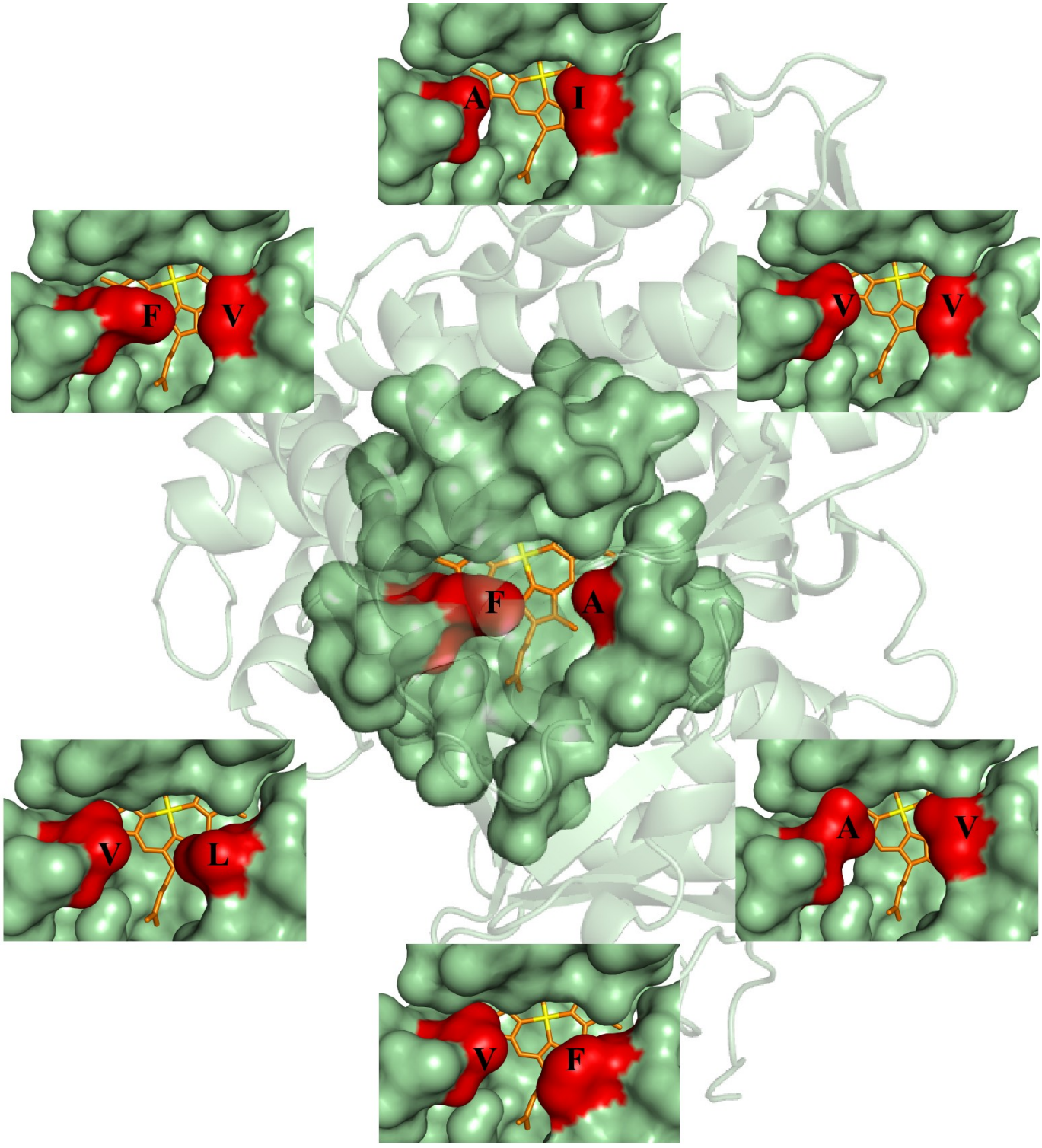
**Sometimes it needs no more than a single mutation to make an enzyme convert a substrate that it previously did not interact with. There is an art to finding an amino acid position in the enzyme that can be mutated and changed in a way that results in the required outcome. But if the researchers succeed in doing so, they are able to 'teach' the enzyme to only convert a specific substrate variant or produce only one specific product variant. The area of white biotechnology benefits from the enzyme's selectivity.**

Enzymes play an economically important role in the field of white biotechnology. They are used as catalysts in the production of fine chemicals, as well as the basic building blocks required in the production of food supplements, the cosmetics industry, drugs and many other substances. Given the nature of enzymes, this can be done both quickly and at low temperatures. The more selectively a specific substrate is converted, the more effective the production of the aforementioned materials. A highly practical and cost-saving strategy is to use substrate mixtures from which the enzyme picks its specific substrate. On the product side, the focus is on the production of molecules that are highly pure and also occur in a specific steric form.

A great deal of experience and specialist knowledge is required to find or design an enzyme that is able to convert a desired substrate either regio- or stereo-selectively. The scientists of the Institute of Technical Biochemistry (ITB) at the University of Stuttgart have both experience and specialist knowledge. They model enzyme molecules for specific applications in industry, for their research partners, and also for use in basic research. With this, they are broadening their range of methods for the design of new molecules and their detailed knowledge about the biochemistry of enzymatic reactions. The group of researchers led by Prof. Dr. Jürgen Pleiss and Dr. Alexander Seifert is mainly focused on lipase and monooxygenase enzymes.

## Enzyme selectivity is the specialty of the ITB researchers

Dr. Pleiss spoke about his work. "We carry out regioselective enzymatic reactions in order to produce flavouring substances for use as food supplements or ingredients for the cosmetics industry. We also produce compounds that can be synthesised into pharmaceutical substances. Carrying out a regioselective reaction refers to a reaction that only takes place at one of several similar molecule regions that would theoretically be suitable for this particular reaction. Highly selective reactions enable us to save costs in the subsequent purification of the products." A growing number of efficient enzyme-catalysed reactions are the key to financially competitive synthesis procedures, and this can be achieved through enzyme design.



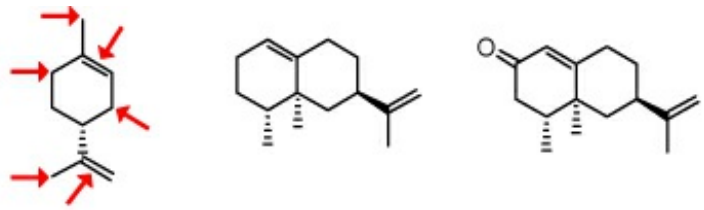

Access to the catalytically active haem group (yellow) in a bacterial monooxygenase enzyme can be modified by introducing mutations into the two “hotspots” (red). The shape of the binding site in six highly selective mutants differs from the binding site of the naturally occurring enzyme (centre). Fig.: Seifert A, Vomund S, Grohmann K, Kriening S, Urlacher VB, Laschat S, Pleiss J: Rational design of a minimal and highly enriched CYP102A1 mutant library with improved regio-, stereo- and chemoselectivity. ChemBioChem. 2009. 10: 853-861. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.


Pleiss uses the example of cytochrome P450 monooxygenases to show that many natural enzyme variants are available. Theses cytochrome monooxygenases occur in all living organisms and are involved in degradation reactions in the liver: "We have around 10,000 different amino acid sequences of this enzyme family stored in our database and know the X-ray structure of around 40 proteins." The ITB team initially designs monooxygenases in silico (i.e. with computers), which are

later produced by bacteria under real conditions. The enzymes convert certain substrates into sought-after products. "Monooxygenase enzymes use molecular oxygen for the oxidative creation of products. In the process of generating a monooxygenase variant, we take great care to maintain the enzyme's basic function and structure. Sometimes, very small differences occur, for example in the substrate binding pockets of the enzyme," said Pleiss.

## The difficulty lies in the detail

These subtle modifications affect an enzyme's selectivity; they may impair or improve an existing reaction, or make a reaction possible that did not previously occur. An identical reaction that occurs at neighbouring C atoms can lead to different products. (R)-limonene for example, a flavouring agent that can be cheaply extracted from oranges. The selective oxidation of (R)-limonene leads to valuable products. However, the traditional chemical oxidation of this terpene results in a mixture of different products. "In order to obtain a pure product, purification steps are necessary, which of course, increases the production costs. In addition, material is lost and side products need to be disposed of. Therefore, the price of the final product is several times higher than that of the starting substrate," said Pleiss.



Flavouring substances (from left to right): (R)-(+)-limonene, (+)-valencene and (+)-nootkatone. The arrows point towards potential oxidation sites, which are possible targets for enzymatic catalysis.


In many cases the ITB is able to develop an efficient regio- or stereoselective enzyme variant for use in enzymatic production. Sometimes, the label 'bioproduction' is an aspect that plays a major role in developing specific enzyme variants: metal catalysts can convert valencene into nootkatone, the most important and expensive aromatic compound found in grapefruit. "However, the food industry does not want to use heavy metals as catalysts. That is why biocatalysis is an excellent alternative," said Pleiss.

## Learning from nature

First of all, the "right" amino acid, i.e. one that can be mutated to optimise the enzyme's selectivity, needs to be identified. However, this does not mean that this amino acid will be suitable for practical application. Dr. Alexander Seifert explains: "An important aspect is the variability of the amino acid. We do not want to change highly-conserved amino acids, which are vital for the enzyme molecule's overall structure. It is our aim to identify as few as possible amino acids that are suitable for mutation." Pleiss adds: "We try to induce minimal change." The team bases its methods on the example of nature: "Certain amino acid exchanges are also found in nature. We take this to mean that amino acid exchanges are a natural phenomenon," said Seifert.

There are other research groups in Germany and around the world that focus on the design of the same groups of enzymes. The ITB group believes that it is at an advantage as its systematic

approach is not yet widely known. “We not only model enzymes, but also show directly in the laboratory that the enzymes we design actually work. We are able to produce milligrams of a certain enzyme in our laboratories,” said Pleiss, going on to add that many approaches used by his team do not focus on obtaining a particular result. The researchers are not looking for an enzyme that is ideal for a certain type of production, but they carry out promising enzyme mutations, which are then analysed to find out which substrates are converted into which products. Interesting results then enable the researchers to focus systematically on the further optimisation of enzyme design.

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**Article**

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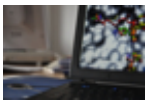

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